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SHORT COMMUNICATION

Detection of Zika virus in *Aedes* mosquitoes from Mexico

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Background: We report on the results of an entomovirological surveillance system of *Aedes* populations performed by the Ministry of Health of the central state of San Luis Potosí, Mexico.

Methods: Indoor adult *Aedes aegypti* and *Aedes albopictus* pools collected at San Martín, Tamazunchale, Ciudad Valles, Metlapa, Ebano, Tamuin and Axtla during the dry season of 2016 were examined for the presence of dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) viruses using real-time PCR.

Results: Both *Ae. aegypti* and *Ae. albopictus* were found to be infected with ZIKV in the absence of confirmed symptomatic human cases.

Conclusions: The entomovirological surveillance system analysed here identified both *Ae. aegypti* and *Ae. albopictus* infected with ZIKV which triggered an immediate aggressive vector control campaign.

Keywords: *Aedes aegypti*, *Aedes albopictus*, Dengue, Mexico, Surveillance, ZIKV

Background

Aedes aegypti and *Aedes albopictus* are present in Mexico. *Aedes aegypti* is prevalent in all states of Mexico (except Tlaxcala)[1] and *Ae. albopictus* is mainly found in the states of the Gulf of Mexico (except in Yucatán and Campeche), in the states of the Pacific coast (Chiapas, Oaxaca and Sinaloa) and also in several states of central Mexico such as Morelos, Hidalgo and San Luis Potosí.[2] *Aedes aegypti* is the primary vector associated with current dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) viruses in Mexico. Although *Ae. albopictus* has been found infected with all the four serotypes of DENV in Mexico, there are only a few studies on the presence of other arboviruses in wild populations of this species in Mexico. Here, we report evidence of the presence of ZIKV in *Ae. aegypti* and *Ae. albopictus* collected in the central state of San Luis Potosi, Mexico.

Methods

Samples of resting adult female *Ae. aegypti* and *Ae. albopictus* were collected (indoors and outdoors in houses and in cemeteries) for 15 min periods with Prokopack aspirators[3] during the dry season of 2016 (February–March) in 199 houses (randomly chosen)

and three cemeteries from seven towns of the Mexican central state of San Luis Potosi—San Martín (19 houses), Tamazunchale (20 houses), Ciudad Valles (31 houses and two cemeteries), Matlapa (24 houses and one cemetery), Ebano (38 houses), Tamuin (51 houses) and Axtla (16 houses) (Figure 1). The choice of towns was based on their historically high levels of DENV transmission. In each town, the selection of the area for adult female *Aedes* collections was guided by entomological (ovitrapping) and epidemiological information stored in the National Database for Dengue Surveillance (https://www.gob.mx/cms/uploads/attachment/file/23789/Lineamientos_para_la_vigilancia_epidemiologica_de_dengue.pdf). Within a given week, city blocks from each locality that had high entomological risk (those reporting an average number of *Aedes* spp. eggs in the upper fourth quartile of the distribution of eggs per trap within a network of ovitraps evenly located within each town) were chosen for female *Aedes* adult collections. All sample collections occurred during a period of 15 min per house between 09:00 and 15:00 h following a published procedure.[4] Collected specimens were initially separated by species in situ by skilled field entomologists due to the fact that both *Ae. aegypti* and *Ae. albopictus* species are present in the area (Table 1). Individual samples by species/house were vialed together with BD Universal Viral Transport medium (Becton, Dickinson Company East Rutherford, NJ, USA), kept at 4–8°C to avoid degradation of the genetic material and sent to the National Reference Laboratory [Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE)] for species identification confirmation and a further arbovirus detection process following standard operational procedures.[3] Briefly, adult female mosquitoes were grouped in pools of 4 up to 25 (mean=9.3) specimens. Each pool was homogenized with a tissue disruptor (QIAGEN, Inc., Valencia, CA, USA) and centrifuged at 2700 rpm for 10 min at 4°C. RNA was extracted and purified from the supernatant using a QIAamp Viral RNA minikit (QIAGEN and stored at −20°C until further use. Each pool was processed to test for the presence of DENV, CHIKV and ZIKV using existing real-time RT–PCR protocols (https://www.gob.mx/cms/uploads/attachment/file/220404/Lineamientos_ve_y_lab_virus_fiebre_Chikungunya.pdf). To validate our results, a positive control (synthetic RNA from a reference strain available at InDRE) and a negative control were included in each RT-PCR run. After the amplification protocol, curves were evaluated and the threshold line placed above background signal, with a detection limit of ≤39 cycles.[5] The sequences of primers for ZIKV detection by real-time PCR were:

- *Primer Forward*: 5′ CCG CTG CCC AAC ACA AG 3′;
- *Primer Reverse*: 5′ CCA CTA ACG TTC TTT TGC AGA CAT 3′;
- *Probe*: 5′ TEXAS RED-AGC CTA CCT TGA CAA GCA GTC AGA CAC TCA A -BHQ1 3′.

The RT-PCRs were performed using the SuperScript III Platinum One-Step Quantitative RT-PCR System without Rox (Invitrogen, cat# 11732-088, Staley Road Grand Island, NY, USA).

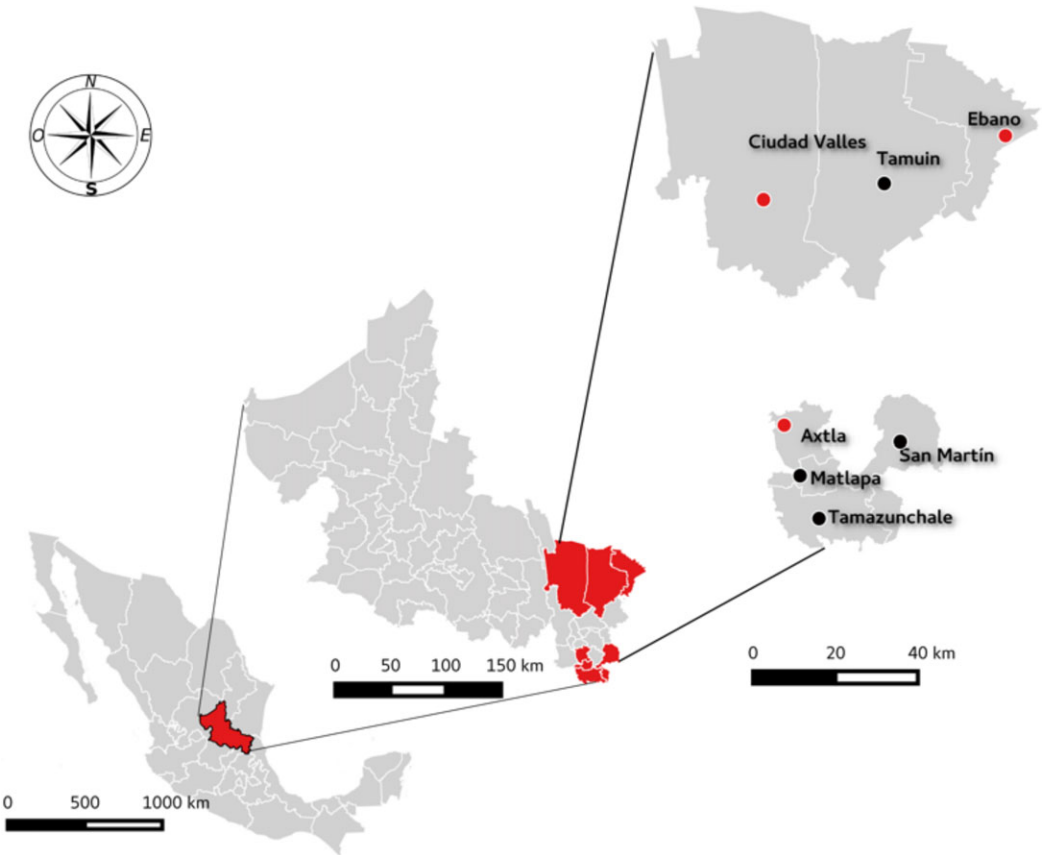

Figure 1. Location of the Mexican Central State of San Luis Potosí and the Municipalities where *Ae. albopictus* samples were collected.

Table 1. Summary of female *Aedes* collections in San Luis Potosí, Mexico and results for ZIKV detection

Species	Town	Total samples (positive)	Date	Number of tested pools (specimens)	Number of positive pools	Minimum infection rate (95% CI)	
Ae. aegypti	Ciudad Valles	2 (1)	9/03/16	1 (5)**	1	N/A*	42.31 (8.21–151.96)
	Ebano	14 (3)	17/02/16	1 (12)	0	29.32 (2.06–232.71)	
		12 (3)	16/03/16	1 (21)	1		
	Tamuin	10 (3)	17/02/16	1 (6)	0	0	
		28 (3)	9/03/16	1 (10)	0		
Ae. albopictus	San Martin	11 (4)	10/02/16	1 (6)	0	0	15.72 (0.99–75.66)
		8 (2)	9/03/16	1 (4)	0		
	Ciudad Valles	7 (3)	10/02/16	1 (25)	0	0	
	Matlapa	12 (2)	17/02/16	1 (5)	0	0	
	Tamazunchale	8 (2)	10/02/16	1 (6)	0	0	
		8 (2)	9/02/16	1 (5)	0		
	Axtla	16 (2)	16/03/16	1 (10)	1	N/A*	
Total		136 (30)		12	3		

* When all pools are positive, the likelihood methods fail. Likelihood estimates therefore do not exist in this case, indicated as N/A for these quantities.
** Sample from a cemetery.

For RT-PCR, each reaction containing 5 μL of RNA was mixed with the following reagents: 6.35 μL of nuclease-free H_2O, 12.5 μL of 2X pre-mix, 0.25 μL of forward and reverse primers for ZIKV (final concentration 1 μM), 0.45 μL of Taqman probe (final concentration 50 nM) and 0.5 μL of SuperScript III RT/Platinum Taq mix to a final reaction volume of 25 μL. Each reaction was run in either 8-tube optical strips or 96-well plates and placed in the CFX96 thermocycler (BIORAD, Alfred Nobel Drive Hercules, California, CA, USA). The standard cycling method was selected and a fluorescence capture set was used to detect emissions through the Texas Red channel in each well. Thermocycling parameters were as follows: reverse transcription (RT) at 50°C for 30 min, RT inactivation at 95°C for 2 min and fluorescence detection for 45 cycles at 95°C for 15 s and an annealing step at 60°C for 1 min. The reactions were validated with synthetic positive control produced at InDRE [GenBank reference: KU556802.1].

Results and discussion

From the total pools processed, 25% (3/12) were positive for ZIKV; these were collected from 42.8% (3/7) of the localities (two households and one cemetery). A total of 2/5 pools of *Ae. aegypti* females and 1/7 pool of *Ae. albopictus* were positive for ZIKV. One of the ZIKV-infected *Ae. aegypti* pools was collected in a cemetery, whereas the remaining positive pools were from residences. The overall ZIKV minimum infection rates (MIRs) were 42.31 [95% confidence interval (CI) 8.21–151.196] for *Ae. aegypti* and 15.72 (95% CI 0.99–75.66) for *Ae. albopictus*.

The sensitivity of RT–PCR is less than 25 genome copies/μL. The Ct value and genome copies obtained for positive pools were:

- *Ciudad Valles*: Ct: 36.41 and genome copies: 1.35/μL;
- *Ebano*: Ct: 32.97 and genome copies: 1.99/μL;
- *Axtla*: Ct: 35.40 and genome copies: 1.43/μL.

The positive pools were isolated in Vero E6 cell line ATCC CRL 1586 (Manassas, VA, USA) with 10% fetal calf serum (GIBCO, Grovemont Cir, Gaithersburg, MD, USA) and confirmed by real-time PCR.

Immediately after the detection of ZIKV-infected mosquitoes, the Mexican Government started an aggressive vector control campaign in San Luis Potosi according to the national protocol (https://www.gob.mx/salud/acciones-y-programas/guias-operativas.html), both at citywide and foci level, that included the control of breeding sites and indoor/outdoor chemical control of adult female mosquitoes. Breeding sites were eliminated and/or treated with a larvicide (spinosad, Natular DT 7.48%, Clarke Mosquito Control, Rosella, IL, USA).

Focal chemical adult mosquito control (within an area of eight blocks containing the positive spot) included: outdoor thermal-fogging (chlorpyrifos 13.6%, MosquitoMist™ ONE U.L.V., Clarke Mosquito Control, Rosella, IL, USA, applied with a SwingFog SN81, Swingtec GmbH, Isny, Germany) and indoor spraying (deltamethrin at 25%, Deltamethrin WG 25, Bayer CropScience AG, Monheim am Rhein, Germany, applied with SOLO PORT 423, Solo Kleinmotoren GMbH, Sindelfingen, Germany). Every locality received a cycle of ULV spraying (malathion 40%, Lethal Mist 44 EW, Cheminova, Harboøre, Denmark, applied with a Leco 1800E ULV Cold Fog Generator, Clarke Mosquito Control) from vehicles.

We are aware of several limitations inherent to this observational study. This was a cross-sectional study that prevented calculating temporal variability in infection rates as the CHIKV epidemic unveiled; in addition, the low viral load did not allow virus characterization by sequencing.

The entomovirological surveillance can be a useful tool in the surveillance of diseases with a high proportion of asymptomatic patients such as Zika, and permits the detection of the

circulation of ZIKV before human cases are confirmed. At that time, none of these localities had confirmed human ZIKV infections and none of them confirmed ZIKV infections in humans for 5 months. In fact, the first detected cases of Zika-infected humans in San Luis Potosi were reported at the end of 2016. An additional important fact is that nine municipalities of San Luis Potosi state are now affected by ZIKV, placing this state above the national mean of ZIKV positivity (38% vs. 25%). This study demonstrates that the early detection of ZIKV-infected mosquitoes followed by prompt vector control actions could have been an important factor in containing virus transmission. In the short term, we will incorporate in Mexico the routine detection of other vector species and more arboviruses of public health importance (West Nile virus, Saint Louis encephalitis virus, Venezuelan encephalitis virus, Western encephalitis virus, East encephalitis virus, Yellow Fever virus, Mayaro virus).

Authors' contributions: All authors read and approved the final manuscript. JADQ wrote the paper, and is the lead scientist, HH, JFG-R, GS-T, FC-M, FER-C, RC-F, MLR-M, JMM-R, JJS-M, GMV-P, PM-S, FD-M, MV-P, CR-J, MdLT-R and AN-L, designed the experiments, collected the samples and conducted the experiments. BT-L, IL-M, CR-M and PAK-M analyzed the data, designed the discussion and provided the logistic support.

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Competing interests: None declared.

Ethical approval: Not required.

References

1 Kuri-Morales P, Correa-Morales F, González-Acosta C et al. First report of *Stegomyia aegypti* (= *Aedes aegypti*) in Mexico City, Mexico. Med Vet Entomol 2017;31:240–2.

2 Ortega-Morales AI, Rodríguez QK. First record of *Aedes albopictus* (Diptera: Culicidae) in San Luis Potosí, México. J Vector Ecol 2016;41:314–15.

3 Díaz-Quiñónez JA, Escobar-Escamilla N, Wong-Arámbula C et al. Asian genotype Zika virus detected in traveler returning to Mexico from Colombia, October 2015. Emerg Infect Dis 2016;22:937–9.

4 Vázquez-Prokopec GM, Galvin WA, Kelly R, Kitron U. A new, cost-efective, battery-powered aspirator for adult mosquito collections. J Med Entomol 2009;46:1256–9.

5 Díaz-Quiñonez JA, López-Martínez I, Torres-Longoria B et al. Evidence of the presence of the Zika virus in Mexico since early 2015. Virus Genes 2016;52:855–7.